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Europium-sensitized and simultaneous pH-assisted spectrofluorimetric assessment of ciprofloxacin, norfloxacin and gatifloxacin in pharmaceutical and serum samples

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ABSTRACT

A facile, ultrasensitive and interference free europium-sensitized spectrofluorimetric method was developed for the simultaneous determination of ciprofloxacin (CFLX), norfloxacin (NFLX), and gatifloxacin (GFLX) in their mixture. A coordination complexes of Eu^{3+}–CFLX/NFLX/GFLX were formed and the coordination was determined using the mole ratio method of continuous variation of equimolar solutions. It was found that the luminescence intensity of Eu^{3+} for these complexes in their mixtures is quite sensitive to three variant sets of pH, 6.0, 5.5 and 3.5 and excitation wavelengths (λ_{ex}), 365, 340 and 395 for CFLX, NFLX and GFLX, respectively. Thus a dual-controlled luminescence of smoothly dynamic reversibility is achieved where a reversible on/off switchable emission of one system was observed by tuning its optimal values of pH and λ_{ex} to the optimal ones of the second and so on for the third. The monitored luminescence intensity of the system showed a good linear relationship with the concentration of CFLX over the range of 5×10^{-8} to 1×10^{-6} mol L^{-1} with a correlation coefficient of 0.995, for NFLX within a range of 5×10^{-8} to 1.8×10^{-6} mol L^{-1} with a correlation coefficient of 0.990, and for GFLX within a range of 5×10^{-8} to 1.2×10^{-6} mol L^{-1} with a correlation coefficient of 0.995. The detection limit (LOD) was determined as 1.5×10^{-8} mol L^{-1} for CFLX, 3.0×10^{-8} mol L^{-1} for NFLX and 1.6×10^{-8} mol L^{-1} for GFLX. The limit of quantification (LOQ) is 4.5×10^{-8}, 9.0×10^{-8}, 2.8×10^{-8} for CFLX, NFLX and GFLX, respectively. Moreover, a ternary mixture of CFLX, NFLX and GFLX was satisfactorily assessed with average error <4.5%. This method has been successfully validated to the simultaneous and selective determination of CFLX, NFLX and GFLX in pharmaceuticals and serum samples with recoveries of (100.2 ± 2.1, 99.6 ± 2.5 and 103.1 ± 2.9) and (102.9 ± 1.1, 102.5 ± 1.5 and 104.4 ± 3.9), respectively.

1. Introduction

Quinolones are broad spectrum synthetic antimicrobial agents. They could be used to inhibit the action of bacterial DNA gyrase enzymes, thus preventing DNA replication [1]. They are classified as first, second, third and fourth generation depending on their antibacterial spectrum, potency and pharmacology. The second, third and fourth generations include the fluoroquinolones, which contain a fluorine atom at the C-6 position and a piperazinyl group at the C-7 position and have better antimicrobial activity, as fluorine increases the activity against Gram-positive pathogens including those resistant to β-lactam antibiotics and sulfonamides [2], whereas the piperazino moiety improves the effectiveness against Gram-negative microorganisms. In recent years, fluoroquinolones are widely used in human and veterinary medicine. These compounds are derived from nalidixic acid and anaphthyridine derivative that was introduced for clinical application in livestock and farming industries usually to treat urinary pulmonary and digestive infection [3]. The recent utilization of antibiotics in food producing animals has caused public concern due to the transfer of antibiotic-resistant bacteria to human beings [4].

Ciprofloxacin (CFLX) [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7(piperazinyl)quinolone-3-carboxylic acid], norfloxacin (NFLX) [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7(piperazin-1-yl)quinoline-3-carboxylic acid], and Gatifloxacin (GFLX) [(±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid] (Fig. 1) belong to the fluoroquinolones [5].

CFLX is used in the treatment of systemic infections including urinary tract, respiratory, gastrointestinal and cutaneous infections. Its drug function works by inhibiting both a subunit of

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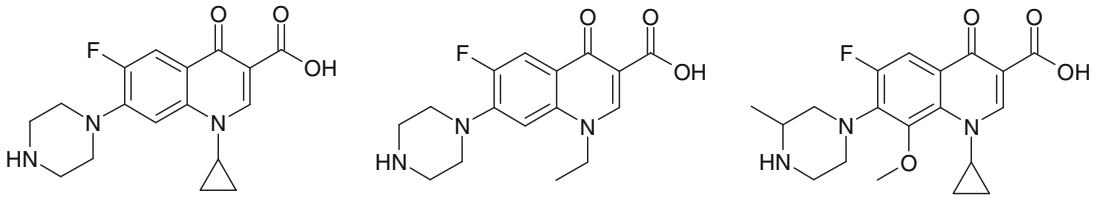

Ciprofloxacin
M. F. = $C_{17}H_{18}FN_3O_3$
M. Wt. = 331.35

Norfloxacin
M. F. = $C_{16}H_{18}FN_3O_3$
M. Wt. = 319.33

Gatifloxacin
M. F. = $C_{19}H_{22}FN_3O_4$
M. Wt. =375.39

Fig. 1. The chemical structure of CFLX, NFLX, and GFLX.

bacterial DNA gyrase (topoisomeraseII) and an essential enzyme that maintains the super helical twists in the bacterial DNA, to affect the growth and reproduction of bacteria [6]. NFLX is the first choice drug for the treatment of diseases caused by *Campylobacter*, *Escherichia coli*, *Salmonella*, *Shigella* and *Vibrio cholerae*. It is used for the treatment of gonorrhoea as well as eye and urinary tract infection [7]. GFLX belongs to the fourth generation of a new class of synthetic antibacterial fluoroquinolone agents [8,9]. Therefore, the widespread use of these compounds and the need for clinical and pharmacological study require fast and sensitive analytical methods for determination of its presence in pharmacological formulations and biological fluids.

Furthermore, fluoroquinolones have suitable functional groups to form stable complexes with Tb^{3+} and Eu^{3+}. The presence of these ions in quinolone solutions leads to the formation of complexes that absorb energy at the characteristic wavelength of the organic ligand and emit radiation of the characteristic wavelength of Tb^{3+} and Eu^{3+}. These complexes show a large Stokes shift and narrow emission bands. This technique has been widely utilized for the determination of quinolones such as; norfloxacin [10], enoxacin [11], garenoxacin [12], trovafloxacin [13], ciprofloxacin [14], gatifloxacin, etc. Other analytical techniques such as spectrophotometry [15–17], chemical luminescence [18], high performance liquid chromatography [19–21], capillary electrophoresis [22–25] and immunosensor [26] were used. However, the fluorometric method has attracted greater attention because of the combination of convenience and sensitivity [27–31].

Compared with most of other methods reported and to date, this work offers the first method for the simultaneous and pH-assisted spectrofluorimetric determination of CFLX, NFLX and GFLX in the same sample in pharmaceuticals. The method implies the formation of coordination complexes between Eu^{3+} and CFLX/NFLX or GFLX. The luminescence intensity of Eu^{3+} for these complexes in their mixtures is quite sensitive to three variant sets of pH and λ_{ex}, thus a dual-controlled luminescence is achieved and a reversible on/off switchable emission of one system was observed by tuning its optimal values of pH and λ_{ex} to the optimal ones of the second and so on for the third. The rapid, facile procedure, high sensitivity, reproducibility of this method opens the door for a more potent simultaneous and interference free analytical approach applications.

2. Experimental

2.1. Apparatus

All luminescence measurements were recorded with a Meslo-PN (222-263000) Thermo Scientific Lumina fluorescence Spectrometer in the range (190–900 nm). The absorption spectra were recorded with a double beam PerkinElmer Lambda 25 UV–vis spectrophotometer employed with a tungsten halogen lamp for operation in the visible range and a deuterium lamp for operation in the UV range. The sample holder was a quartz rectangular cells with 1 cm path length. The pH was measured using with a pHs-JAN-WAY 3330 research pH meter.

2.2. Reagents

A stock standard solution of Eu^{3+} (1.0×10^{-2} mol L^{-1}) was prepared by dissolving 42.8 mg of $Eu(NO_3)\cdot 5H_2O$ (Sigma–Aldrich, purity 99.9%) in 10 mL ethanol. Stock standard solutions (1.0×10^{-2} mol L^{-1}) of CFLX, NFLX, and GFLX (National Institute for the Control of Pharmaceutical and Biological Products) were prepared by dissolving 165.67 mg, 159.67 mg and 187.7 mg of CFLX, NFLX and GFLX, respectively in ethanol and diluting to 50 mL with acetonitrile. All the solutions were kept in the refrigerator and protected from light. Working standard solutions were obtained by making appropriate dilutions of the stock standard solution with acetonitrile.

HCl/KCl buffer 0.2 mol L^{-1} (pH 1.0–2.2) was prepared by mixing appropriate volume of 0.2 mol L^{-1} hydrochloric acid with 0.2 mol L^{-1} potassium chloride. Acetate buffer 0.2 mol L^{-1} (pH 3.5–5.6) was prepared by mixing appropriate volume of 0.2 mol L^{-1} acetic acid with 0.2 mol L^{-1} sodium acetate. Borate buffers (pH 6–8.5) were prepared by mixing appropriate volumes of 0.2 mol L^{-1} boric acid with 0.2 mol L^{-1} sodium hydroxide. Phosphate buffer (pH 8.8–11.0) was prepared by mixing appropriate volume of 0.2 mol L^{-1} K_2HPO_4 with 0.1 mol L^{-1} sodium hydroxide.

Pharmaceutical preparations: CIPROBAY® tablets (Bayer Co.) containing 250 mg CFLX, and NORBACTIN tablets (Ranbaxy, Egypt CO.) containing 400 mg NFLX, in addition to Gatipram eye drops (BETA, Pharma Co.) containing 0.3% GFLX were purchased from local market.

All reagents used were analytical grade unless otherwise indicated. Bidistilled water was used throughout.

2.3. Methods

Working solutions are prepared in 10 mL measuring flasks, where 2×10^{-4} mol L^{-1} of (CFLX) solution, 3×10^{-4} mol L^{-1} of NFLX and GFLX were mixed with 1×10^{-4} mol L^{-1} Eu^{3+} solution then the mixture is diluted to the mark with acetonitrile. The Eu^{3+}–CFLX, Eu^{3+}–NFLX and Eu^{3+}–GFLX systems were adjusted at pH 6.0, 5.5 and 3.5, respectively by using 1 mL, 1.3 mL of borate buffer and 2.3 mL of 0.2 mol L^{-1} of acetate buffer solutions, respectively, and stored for 10–15 min at room temperature. The above procedure is used for the subsequent measurements of absorption, emission spectra, and studying the effect of pH and solvents. The luminescence intensity is measured at $\lambda_{ex}/\lambda_{em}$ = 365/615 nm, 340/617, and 395/617 for Eu^{3+}–CFLX, Eu^{3+}–NFLX and Eu^{3+}–GFLX systems, respectively.